in 2002 and further to 5% in 2011. Overall, the levels of resistance to gentamicin increased from 6.7% in 1981 to 14.8% in 2011 and that to amikacin increased from merely 0.6% in 1981 to 7.2% in 2011.

Conclusion: Despite continuous usage for over three decades, more than 85% of Gram negative bacilli still remain susceptible to gentamicin and amikacin and the compounds continue to remain highly effective in the therapy of common Gram negative infections in this hospital. Similar observations of slow occurrence of resistance to aminoglycoside antibiotics and a decrease of resistance especially in isolates of *Pseudomonas aeruginosa* have been made by others (Clin Infect Dis 19:819,1994., Int J Antimicrob Agents 16:309,2000).

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Genotypic Distribution of Plasmid Encoded Extended Spectrum Beta-lactamases from Lahore, Pakistan

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Background: Successful spread of ESBL can be attributed to the fact that genes encoding for ESBL are often located on self-transmissible or mobilizable broad range plasmids. There is great diversity in type of gene producing beta-lactamase, such as *TEM*, *SHV*, OXA, *PER*, *VEB*, *BES*, *CTX-M* and others. Here genotypic distribution of plasmid encoded extended spectrum Beta-lactamases from Lahore, Pakistan were studied.

Methods: DNA Amplification for *TEM*, *SHV* and *OXA*-1 encoding genes was performed using singlplex and multiplex PCR, plasmid isolation, horizontal gene transfer, isoelectric focuing and amino acids were calculated in local isolates of *E.coli* and *Klebiella* spp.

Results: In 260 clinical isolates of *E. coli* suspected to be ESBL producers OXA (19.2%) and *TEM*/*OXA* (44.2%) were more prevalent. Of the 40 clinical isolates of *Klebsiella* spp 92.5% demonstrated *SHV*-specific products. In 87 environmental isolates of *E. coli* 58.6% *TEM*/*OXA* and out of 13 environmental isolates of *Klebsiella* spp exhibited 76.92% *SHV*. All tested isolates showed high rate of resistance transfer though horizontal gene transfer mechanism. Isolectric focusing was performed that give isoelectric points for specific beta-lactamase.

Conclusion: ESBL plasmid encoded genes were easily transfered through horizontal gene transfer mechanism. The best results were obtained from PCR and DNA sequencing as compared to calculating isolectric points (pI). If somehow multiplex PCR of ESBL will applied in local diagnostic labs this helps in early detection and phenotypic antibiotic therapy against this infection.

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New Delhi metallo-β-lactamase-1 (NDM-1)-producing Enterobacteriaceae: first report in Thailand

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Background: Carbapenems are recommended for treatment of serious infections caused by Gram negative bacilli including ESBL- or AmpC-producing Enterobacteriaceae. However, carbapenem-resistant Enterobacteriaceae by production of carbapenemases have emerged worldwide. In Srinagarind Hospital, ESBL-producing isolates have been found with high prevalence rates in 2010 (46% for *E. coli* and 47% for *K. pneumoniae*), leading to an increasing use of carbapenems. Therefore, surveillance of carbapenemases in clinical isolates of Enterobacteriaceae may contribute useful information for clinician to select appropriate antimicrobial therapy and for infection control to prevent the dissemination of these resistant strains in the hospital.

Methods: A total of 4,818 Enterobacteriaceae isolates collected from patients in Srinagarind Hospital, Thailand between October 2010 and August 2011 were screened for the presence of carbapenemases by ertapenem and imipenem disk diffusion tests. All positive-screening isolates were subjected to modified Hodge test, boronic acid- and EDTA-carbapenem combined disk tests and two multiplex PCR of *bla*IMP, *bla*VIM, *bla*SPM, *bla*SIM and *bla*GIM, and of *bla*KPC, *bla*NDM and *bla*OXA-48. Carbapenemase-producing isolates were typed by ERIC-PCR and REP-PCR and then characterized by antimicrobial susceptibility tests. Conjugation was performed by broth culture mating method.

Results: Six isolates including 2 isolates of each *Escherichia coli*, *Klebsiella pneumoniae* and *Citrobacter freundii* were found to produce NDM-1, whereas other 2 isolates of *K. pneumoniae* produced IMP-14a. DNA fingerprints revealed that the 8 MBL-producing isolates were of different strains. In vitro transfer of carbapenem resistance was successful for all MBL-producing isolates, indicating the ease of transmission of these resistance determinants. MICs of imipenem, meropenem and ertapenem for the NDM-1 producers were 4-16 μg/ml, 4-16 μg/ml and 16-64 μg/ml respectively, whereas those for both IMP-14a producers were 0.25 μg/ml, 0.5-2 μg/ml and 2-4 μg/ml respectively. All MBL-producing isolates were susceptible to colistin and tigecycline. The 6 NDM-1 producers were recovered from urine of three patients, the history of travel outside Thailand was unknown.

Conclusion: Surveillance of these carbapenemases in Enterobacteriaceae is urgently needed to control and prevent the spread of these resistance determinants. The ertapenem disk screening test in combination with the EDTA-carbapenem combined disk test are recommended for MBL detection in routine laboratory.

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